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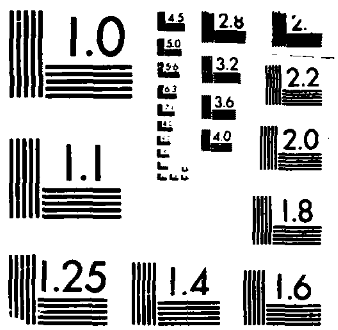

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The long-range goal of this research is to elucidate the molecular mechanism of protein-protein recognition and binding. The immediate goal is to undertake the synthesis and chemical modification of model peptide ligands, to characterize the structure of these peptides through NMR spectroscopy and x-ray crystallography, and to test their function in _in vitro_ and _in vivo_ assay systems. We also propose to identify and characterize the primary and secondary structure of the cell membrane receptor(s) of the ligands. Models of cell membrane receptor-peptide ligand binding will be constructed from structural studies of the purified receptor, NMR analysis of ligand binding to the receptor, and from monoclonal antibody surrogates reacting with the peptide active site.

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PROTEIN STRUCTURE AND FUNCTION: DESIGN AND SYNTHESIS OF PEPTIDE RECOGNITION SEQUENCES

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SUMMARY

The goal of this research is to elucidate the molecular mechanisms of protein-protein recognition and binding. The program includes the synthesis and chemical modification of model peptide ligands, characterization of the structure of these peptides through NMR spectroscopy and x-ray crystallography, and analysis of their function in *in vitro* and *in vivo* assay systems. We also propose to identify and characterize the primary and secondary structure of the cell membrane receptor(s) of the ligands. Models of cell membrane receptor-peptide ligand binding will be constructed from structural studies of the purified receptor, NMR analysis of ligand binding to the receptor, and from monoclonal antibody surrogates reacting with the peptide active site.

1. Research Objectives

Our objectives during the first year of this program have been (a) to synthesize various model AGA peptide ligands, (b) to institute quantitative assays of their activity, (c) to begin the modeling of their structure by NMR spectroscopy and X-ray crystallography, and (d) to begin the isolation and characterization of a protein receptor of the peptide ligands.

2. Summary of Accomplishments, 5/1/87 to 5/1/88

A. Assay of peptide-receptor recognition and binding: A solid phase quantitative assay of peptide-ligand binding to a cellular receptor has been established using microtiter wells coated with fibronectin and HL-60 cells containing ^{51}Cr. Cell binding to the plates is inhibited by synthetic peptides which compete for cell binding to fibronectin. The sensitivity of the assay is in the range of 1 to 1000 ug of inhibitory peptide.

B. Peptide ligands: We have synthesized or obtained to following peptides containing the Arg-Gly-Asp (RGD) sequence and various control peptides for analysis in this system:

GRGDS
GRPDS
GRGDSPC
GRGASPK
GFGDSPC
GRGGSPC
NGRGDST
HHLGGAKQAGDV
GRGDS-amide
GR(D)ADS
FGRGDSAF
PFVRGDSA
YAVTRGDESPASSC

C. Structural analysis: Studies of the structural basis for the specific interactions of peptides and proteins containing the sequence RGD with receptors have been initiated. As a control for future studies of receptor-bound RGD peptides, the solution structure of the pentapeptide GRGDS is being studied by proton NMR. Two-dimensional correlated spectroscopy (COSY) at 250 MHz have permitted the assignments of most of the proton resonances. One- and two-dimensional NOE studies have detected short distances (< 4 A) from Gly_3CH to Asp_4NH, from Ser_5NH to Arg_2CH_2, and possibly from Arg_2C H to Asp_4NH, as well as several intraresidual proximities. These NOEs suggest a partially folded structure with a turn in the R2-G3-D4-S5 region, stabilized by hydrogen bonding between the guanidinium of R2 and either the carboxylate of D4 or the carboxy-terminus of S5. This conformation is similar to one of the low energy conformations for GRGDS calculated by Dr. L. Mario Amzel. The remaining ambiguities will be resolved by adding additional residues to the carboxy-terminus and by completion of assignments at higher magnetic fields (i.e. 600 MHz). Changes in peptide conformation on binding to the receptor are anticipated, and will be studied when sufficient quantities of an R-G-D receptor becomes available.

In preliminary studies of larger peptide ligands, 500 MHz NMR data have been collected on the 14-residue peptide YAVTGRGESPASSC which contains the pentapeptide GRGES, an inactive analog of GRGDS. Structure is detected in the 14-mer by differing chemical shifts of the methyl resonances of Ala_2 and Ala_{11} and of the Cβ methylene resonances of Ser_9, Ser_{12} and Ser_{13}. Two-dimensional NOE studies of the 14-mer and its active analog containing GRGDS will be done at 600 MHz.

D. Receptor purification and characterization: Large scale purification of a receptor protein for the cell adhesion ligand peptides was conducted with the platelet glycoprotein IIbIIIa using affinity chromatography and size exclusion chromatography. Although we readily achieved the purification of large amounts of the protein (100 mg), we were unable to generate active receptor from soluble protein fractions. Since proteins were affinity purified from columns of the 14-residue YAVTRGDESPASSC we speculate that active receptor was present in the proteosomes of the detergent (NP-40) extracts of platelets but that activity was lost in elution of the protein from the affinity support. We have since initiated analysis of other cell proteins that react with the adhesion sequence in experiments to obtain purified active receptor.

3. PLANS FOR NEXT YEAR

Structural analysis by NMR spectrometry and X-ray crystallography will continue with selected peptides, initially with the GRGDS, GRGDS-amide and GRGNS model molecules for which the initial results suggest the presence of low energy conformations appropriate to models of the structure of the molecule. These studies will be followed by the synthesis of other appropriate peptides based upon the prediction of the initial results. Continued research will also be carried out in the purification of a biologically active receptor for these peptides so that we can initiate the analysis of the interaction of the ligands with this receptor.

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